elongating actin filaments reaching a nucleated region can trigger new actin assembly.
Finally, we combined these findings to show how all the studied parameters (geometry, steric interactions, filament rigidity, nucleation efficiency) were necessary for the formation of in-vivo like structures.

2872-Pos Board B564
The Role of Caldesmon and Its Phosphorylation by ERK on the Binding Force of Unphosphorylated Myosin to Actin
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Smooth muscle has the unique property of maintaining tension with low ATP consumption. It is generally accepted that this property, called the latch-state, results from the dephosphorylation of myosin while attached to actin. However, detached dephosphorylated myosin can also bind to actin and contribute to force maintenance. We investigated the effect of caldesmon on the binding force of unphosphorylated myosin to actin. We quantified the average unbinding force (F_{unb}) in the absence or presence of caldesmon, ERK phosphorylated caldesmon, or caldesmon plus tropomyosin. Briefly, a microsphere captured in a single beam laser trap was attached to a fluorescently labeled actin filament that was then brought in contact with a pedestal coated with unphosphorylated myosin. The pedestal was then moved away from the trap at constant velocity. The actin/microsphere followed the pedestal until the force exerted by the trap on the microsphere exceeded the binding force of the unphosphorylated myosin to the actin. At this point, the microsphere sprang back into the trap center. F_{unb} was calculated as the product of the trap stiffness and the maximal displacement of the microsphere from the trap center. F_{unb} was normalized to the number of myosin molecules estimated per actin filament length. F_{unb} from unregulated actin (0.09 ± 0.01 pN) was significantly increased in the presence of caldesmon (0.17 ± 0.02 pN), tropomyosin (0.17 ± 0.02 pN) or both regulatory proteins (0.18 ± 0.02 pN). Interestingly, ERK phosphorylation of caldesmon significantly reduced the F_{unb} (0.06 ± 0.01 pN). Thus, caldesmon enhances the binding force of unphosphorylated myosin to actin potentially contributing to the latch-state. Conversely, ERK phosphorylation of caldesmon decreases this binding force to very low levels, suggesting a mechanism for muscle relaxation from the latch-state.
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Actomyosin Regulation by Tm5NM1, a Non-Muscle Isoform of Tropomyosin
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Actin filaments are required for diverse cellular functions including cell division, intracellular transport, and muscle contraction. Tropomyosin (Tm) is a α-helical coiled-coil protein that regulates its functions and stability in muscle and nonmuscle eukaryotic cells. Nonmuscle isoforms of Tm and particularly, Tm5NM1 (a γTm, product of *TPM3*), have been found to play a role in the transformation and metastasis of cancer cells in addition to being important for normal cellular functions such as cell migration, cell division and organelle transport. In previous work, by making mutations at evolutionarily conserved residues in a striated muscle αTm, residues important for actin binding and myosin regulation were identified. In the present study, we have mutated conserved residues in Tm5NM1 to determine the molecular basis of isoform-specificity for functions such as actin binding and actomyosin regulation. We mutated surface residues in muscle αTm and Tm5NM1 that differed between the two isoforms at homologous positions in periods P1 and P7 of αTm to determine the effect of sequence differences on function. We also mutated residues in Tm5NM1 in periods P5 and P6 at homologous positions to those that were shown to be important for myosin regulation in muscle αTm in a previous study. In vitro motility assays for determining myosin regulation were carried out. Actin-Tm filament velocities were inhibited by αTm (~60%) but activated by Tm5NM1 (~50%) relative to actin alone. The αTm P1 and P7 mutants showed ~40-70% increase in velocity compared to WT αTm. The Tm5NM1 mutants had no effect on velocity relative to WT Tm5NM1, except the P6 mutant that showed a ~50% decrease in velocity. Therefore, αTm and Tm5NM1 have differential effects on actomyosin regulation depending on the site of mutation. *Supported by NIH and Aresty Research Center at Rutgers.*

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Cofilin Binding to Globular and Filamentous Actin
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Cofilin is important for the regulation of the actin cytoskeleton. It is capable of binding to and severing F-actin filaments, but the molecular basis for these functions is poorly understood, due to a paucity of structural data on the quaternary complex. A recent computational model has been proposed (Galkin et al., 2011, PNAS, 108:20568). To test this model, we inserted mutant residues into the sequence of cofilin to facilitate specific labelling with spectroscopic probes. We were able to successfully mutate and express four mutant cofilins. We were hampered in this process by the apparent sensitivity of the structure of cofilin to mutagenesis. Thus, we found that mutagenesis of the N-terminus abolished G-actin binding, while still permitting F actin binding, reinforced the importance of the N-terminus of cofilin in binding to actin. On the other hand, mutation of the C-terminus of cofilin and W135 had no effect on the binding of cofilin to G-actin. Mutation of W104 abolished G-actin binding, highlights the importance of the β5 strand and α6 helix of cofilin in actin binding via the G/F actin binding site on cofilin. Fortunately, the mutant forms of cofilin that retained binding to G actin (Cys170 cofilin and Cys170 W104 cofilin) retained their capacity to bind to G-actin after modification with extrinsic spectroscopic probes, allowing us to measure one distance within cofilin and three distances between cofilin and G-actin. The distances between cofilin and G-actin corresponded closely to the predicted distances from the Galkin model.

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Functional Differences of Unbound and Weakly Bound Xb States in the Cardiac Myofilament
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Background: Ca^{2+} and cross-bridges are involved the activation and deactivation of the cardiac actin filament. The actin filament is decorated with a repeating lattice of Tn and Tm. The affinity of rhodamine-phalloidin for actin is sensitive to the twist of f-actin.
Experimental design: (1) Murine cardiac myofibrils were incubated with an excess of dye-labeled cTn under conditions that favor strongly bound (no nucleotide, ADP, ADP + blebbistatin), weakly bound (ADP + Pi, ADP + Pi + blebbistatin), or unbound (ATP, ATP-γ-S) myosin. (2) Rigor and ATP-saturated myofibrils were stained with rhodamine-phalloidin. Samples were immunostained for α-actinin examined by epifluorescence.
Results: Strongly-bound and weakly-bound myosin promotes cTn exchange near the z-disc. Unbound myosin promotes cTn exchange near the M-line. The site of cTn exchange correlated with phalloidin binding.
Conclusions: The native actin filament exhibits region-dependent stability subject to filament-wide perturbation by strongly-bound and weakly-bound myosin motors.
Significance: The native cardiac actin filament has unique functional properties not present in solution studies.

2876-Pos Board B568
Binding of Nucleocapsid Domain of HIV Gag Protein to Actin
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There has been a growing interest in the role of actin in the pathway of HIV infection: entry of HIV into cells involves a hijacking of the actin cytoskeleton, and nuclear migration of the virus requires actin. It was shown that the nucleocapsid domain (NC) of retroviral Gag protein can be associated with F-actin in a dose-dependent fashion in vitro and suggested that the interaction between HIV Gag and the actin cytoskeleton through the NC domain may play an important role in HIV assembly (Iyengar et al., 1998; Liu et al., 2009). However, the specificity of this interaction has never been established.
We have used electron microscopy and the IHRSR method to reconstruct actin filaments decorated with the NC domain of HIV-1. We see strong and specific binding of this 55 residue NC domain to F-actin. Work is in progress to bring the resolution of this complex to the near-atomic level of resolution that we have now achieved for pure F-actin using a Titan Krios robotic microscope equipped with a direct electron detector. Exploring the interaction of HIV NC with actin could open up completely new areas in understanding HIV pathogenesis as well as in developing new drug targets.